TOXICOLOGICAL SCIENCES 123(1), 290–303 (2011)
doi:10.1093/toxsci/kfr142
Advance Access publication June 24, 2011

Perfluoroalkyl Sulfonates Cause Alkyl Chain Length–Dependent Hepatic Steatosis and Hypolipidemia Mainly by Impairing Lipoprotein Production in APOE*3-Leiden CETP Mice

Silvia Bijland,*,[1] Patrick C.N. Rensen,†,[1] Elsbet J. Pieterman,‡ Annemarie C.E. Maas,‡ José W. van der Hoorn,‡ Marjan J. van Erk,§ Louis M. Havekes,†,‡,¶ Ko Willems van Dijk,*,† Shu-Ching Chang,|| David J. Ehresman,|| John L. Butenhoff,|| and Hans M.G. Princen‡,[2]

*Department of Human Genetics and †Department of General Internal Medicine, Endocrinology, and Metabolic Diseases, Leiden University Medical Center, 2300 RC Leiden, The Netherlands; ‡Gaubius Laboratory, Department of Biosciences, Netherlands Organization for Applied Scientific Research—Quality of Life, 2301 CE Leiden, The Netherlands; §Netherlands Organization for Applied Scientific Research—Quality of Life, 3700 AJ Zeist, The Netherlands; ¶Department of Cardiology, Leiden University Medical Center, 2300 RC Leiden, The Netherlands; and ||Medical Department, 3M Company, St Paul, Minnesota 55144-1000

[1]These authors contributed equally to this study.
[2]To whom correspondence should be addressed at Gaubius Laboratory, Department of Biosciences, Netherlands Organization for Applied Scientific Research—Quality of Life, PO Box 2215, 2301 CE Leiden, The Netherlands. Fax: +31 715181901. E-mail: hans.princen@tno.nl.

Received March 31, 2011; accepted May 20, 2011

Perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) are stable perfluoroalkyl sulfonate (PFAS) surfactants, and PFHxS and PFOS are frequently detected in human biomonitoring studies. Some epidemiological studies have shown modest positive correlations of serum PFOS with non–high-density lipoprotein (HDL)-cholesterol (C). This study investigated the mechanism underlying the effect of PFAS surfactants on lipoprotein metabolism. APOE*3-Leiden.CETP mice were fed a Western-type diet with PFBS, PFHxS, or PFOS (30, 6, and 3 mg/kg/day, respectively) for 4–6 weeks. Whereas PFBS modestly reduced only plasma triglycerides (TG), PFHxS and PFOS markedly reduced TG, non-HDL-C, and HDL-C. The decrease in very low-density lipoprotein (VLDL) was caused by enhanced lipoprotein lipase-mediated VLDL-TG clearance and by decreased production of VLDL-TG and VLDL-apolipoprotein B. Reduced HDL production, related to decreased apolipoprotein AI synthesis, resulted in decreased HDL. PFHxS and PFOS increased liver weight and hepatic TG content. Hepatic gene expression profiling data indicated that these effects were the combined result of peroxisome proliferator–activated receptor alpha and pregnane X receptor activation. In conclusion, the potency of PFAS to affect lipoprotein metabolism increased with increasing alkyl chain length. PFHxS and PFOS reduce plasma TG and total cholesterol mainly by impairing lipoprotein production, implying that the reported positive correlations of serum PFOS and non-HDL-C are associative rather than causal.

Key Words: cholesterol; lipoprotein; perfluorooctane sulfonate; PPARα; PXR; triglyceride.

Perfluoroalkyl sulfonates (PFAS) including perfluorobutane sulfonate (PFBS), perfluorohexane sulfonate (PFHxS), and perfluorooctane sulfonate (PFOS) are exceptionally stable surfactant molecules that are representative members of the PFAS class of perfluoroalkyls. Due to their unique physical and chemical properties, PFAS have been used, either directly or by use of N-alkyl functionalized perfluoroalkyl sulfonamides, in industrial and consumer products with applications requiring stability toward corrosion and heat, strong surface tension reduction, and resistance to water and oil. Applications have included paper and textile coatings, food packaging, surfactants, repellents, and fire-retardant foams (Calafat *et al.*, 2007; Kissa, 2001). Although used directly as surfactants or ion-pairing agents, these molecules can also result from metabolic (Xu *et al.*, 2004, 2006) or environmental (D'eon *et al.*, 2006) degradation of *N*-substituted perfluoroalkyl sulfonamides.

In 2001, the widespread distribution of PFOS and PFHxS in humans and PFOS in wildlife was reported (Giesy and Kannan, 2001; Hansen *et al.*, 2001). Since that time, due to their structural stability, widespread dissemination in the environment, and poor elimination in most species, particularly humans (Olsen *et al.* 2007), PFHxS and PFOS have been detected frequently in biological and environmental matrices (Lau *et al.*, 2007). These findings led the major U.S. manufacturer, 3M Company, to discontinue manufacture of PFOS, PFHxS, and materials that could generate these compounds via degradation by the end of 2002. International regulatory action has been taken to further restrict the use of PFOS and materials that may generate PFOS (Lau *et al.*, 2007). PFBS has a more favorable toxicological and environmental profile than either PFHxS or PFOS. In fact, the geometric mean serum elimination half-lives of PFOS and PFHxS determined for 26 retired production workers were, in years, 4.8 (95% confidence interval [CI] 4.0–5.8) and 7.3 (95% CI 5.8–9.2), respectively (Olsen *et al.*, 2007). In contrast, PFBS had a serum

elimination half-life of 25.8 days (95% CI 16.6–40.2) among six production workers removed from exposure (Olsen *et al.*, 2009). Several epidemiological studies have shown modest positive correlations of serum lipids with serum PFOS in nonoccupationally exposed populations (Nelson *et al.*, 2010; Steenland *et al.*, 2009) as well as some occupationally exposed populations (Olsen *et al.*, 2003), although no correlations of serum PFOS with high-density lipoprotein (HDL)-cholesterol (C) have been noted (Nelson *et al.*, 2010; Olsen *et al.*, 2003). However, it is still unclear whether these correlations are causal or associative.

In toxicological studies using cynomolgus monkeys (Seacat *et al.*, 2002), rats (Curran *et al.*, 2008; Haughom and Spydevold, 1992; Martin *et al.*, 2007), and pregnant mice (Manal *et al.*, 2009), PFOS was shown to reduce serum total cholesterol (TC) and otherwise induce changes in plasma lipid metabolism and to increase liver triglycerides (TG). PFHxS was also shown to reduce serum TC in rat (Butenhoff *et al.*, 2009), and PFBS was not (Lieder *et al.*, 2009). However, the mechanisms underlying the effects of PFBS, PFHxS, and PFOS on lipoprotein metabolism, as well as the importance of the alkyl chain length to these effects, have not been addressed thoroughly. Thus, the objective of this study was to investigate the mechanisms underlying the effects of PFHxS and PFOS on lipoprotein metabolism and to assess the importance of the alkyl chain length by comparing the effects between PFBS and PFHxS and PFOS. APOE*3-Leiden.CETP (E3L.CETP) mice (Westerterp *et al.*, 2006) were used, which have attenuated clearance of apoB-containing lipoproteins and exhibit a human-like lipoprotein metabolism on a Western-type diet.

MATERIALS AND METHODS

Ethics statement. The institutional Ethical Committee on Animal Care and Experimentation of TNO-Quality of Life approved all experiments (permit number DEC2483).

Perfluoroalkyl sulfonates. Test materials were provided by 3M Company (St Paul, MN). Potassium PFBS (L-7038, 98.2% pure) contained 1.799% impurities, of which 0.9% detected by nuclear magnetic resonance (NMR), 0.774% detected by liquid chromatography–mass spectrometry (LC-MS), 0.004% metal impurities, 0.101 anionic impurities, and 0.02% residual solvents by thermogravimetric analysis. Potassium PFHxS (L-9051, 99.98% pure) contained 0.021% total identified impurities. Specific isomer concentrations as determined by ^{19}F-NMR were as follows: linear 88.93%, terminal isopropyl branched 6.83%, γ internal branched 2.75%, α internal branched 0.83%, β internal branched 0.56%, and terminal isobutyl branched 0.078%. Identified fluorochemical impurities and their concentrations by LC-MS were $C_6F_{12}HSO_3^-$ K^+ 0.009%, $C_7F_{15}SO_3^-$ K^+ 0.006%, and $C_4F_9SO_3^-$ K^+ 0.004%. In addition, there were < 0.0034% probable C_nH_{2n+2} hydrocarbons < 0.0034% as determined by ^{1}H-NMR. Potassium PFOS (FC-95, 87.6% pure) is from a representative lot that has been used for many toxicological studies (Lot 217) and has previously been described in detail (Seacat *et al.*, 2002).

Animals. In this study, male E3L.CETP mice on a C57Bl/6 background (Westerterp *et al.*, 2006) were used, housed under standard conditions in conventional macrolon cages (two to four mice per cage, wood dust bedding) with free access to food and water. At the age of 8–10 weeks, mice were fed a semisynthetic Western-type diet, containing 0.25% (wt/wt) cholesterol, 1% (wt/wt) corn oil, and 14% (wt/wt) bovine fat (Hope Farms, Woerden, The Netherlands) for 4 weeks in three independent experiments. Upon randomization according to body weight, plasma TC, and TG levels, mice received the Western-type diet without or with PFBS (0.03%, ~30 mg/kg/day), PFHxS (0.006%, ~6 mg/kg/day), or PFOS (0.003%, ~3 mg/kg/day) for 4–6 weeks, based on unpublished pilot experiments in E3L mice. Experiments were performed after 4 h of fasting with food withdrawn at 8:00 A.M.

Determination of serum concentrations of PFBS, PFHxS, and PFOS. Serum concentrations of PFBS, PFHxS, and PFOS were determined using an LC-MS/MS method as described (Ehresman *et al.*, 2007).

Plasma lipid and lipoprotein analysis. Plasma was obtained via tail vein bleeding and assayed for TC and TG, using the commercially available enzymatic kits 236691 and 11488872 (Roche Molecular Biochemicals, Indianapolis, IN), respectively. Free fatty acids (FA) were measured using NEFA-C kit from Wako Diagnostics (Instruchemie, Delfzijl, the Netherlands) and glycerol was measured using the free glycerol determination kit (Sigma-Aldrich, St Louis, MO). The distribution of lipids over plasma lipoproteins was determined using fast protein liquid chromatography. Plasma was pooled per group, and 50 µl of each pool was injected onto a Superose 6 PC 3.2/30 column (Äkta System, Amersham Pharmacia Biotech, Piscataway, NJ) and eluted at a constant flow rate of 50 µl/min in PBS, 1mM EDTA, pH 7.4. Fractions of 50 µl were collected and assayed for TC as described above. HDL was isolated by precipitation of apoB-containing lipoproteins from 20 µl EDTA plasma by adding 10 µl heparin (LEO Pharma, The Netherlands; 500 U/ml) and 10 µl 0.2M $MnCl_2$. The mixtures were incubated for 20 min at room temperature and centrifuged for 15 min at 13,000 revolutions per minute (rpm) at 4°C. HDL-C was measured in the supernatant using enzymatic kit 236691 (Roche Molecular Biochemicals). Plasma cholesteryl ester transfer protein (CETP) mass was analyzed using the CETP ELISA kit from ALPCO Diagnostics (Salem, NH). Plasma apoAI concentrations were determined using a sandwich ELISA (van der Hoorn *et al.*, 2008), with diluted mouse plasma (dilution 1:400,000). Purified mouse apoAI from Biodesign International (Saco) was used as a standard.

In vivo *clearance of VLDL-like emulsion particles.* Glycerol tri[^{3}H]oleate (triolein [TO])-labeled VLDL-like emulsion particles (80 nm) were prepared as described by Rensen *et al.* (Rensen *et al.*, 1997). In short, radiolabeled emulsions were obtained by adding 100 µCi of [^{3}H]TO to 100 mg of emulsion lipids before sonication (isotopes obtained from GE Healthcare, Little Chalfont, UK). Mice were fasted for 4 h, sedated with 6.25 mg/kg acepromazine (Alfasan), 6.25 mg/kg midazolam (Roche), and 0.3125 mg/kg fentanyl (Janssen-Cilag) and injected with a large bolus of radiolabeled emulsion particles (1.0 mg TG in 200 µl PBS) via the tail vein. At indicated time points after injection, blood was taken from the tail vein to determine the serum decay of [^{3}H]TO. At 30 min after injection, plasma was collected by orbital puncture and mice were sacrificed by cervical dislocation. Organs (i.e., liver, heart, perigonadal fat, spleen, and skeletal femoralis muscle) were harvested and saponified in 500 µl Solvable (Perkin-Elmer, Wellesley, MA) to determine [^{3}H]TO uptake. The half-life of VLDL-[^{3}H]TO was calculated from the slope after linear fitting of semi-logarithmic decay curves.

Hepatic lipase and lipoprotein lipase assay. Lipolytic activity of both lipoprotein lipase (LPL) and hepatic lipase (HL) was determined as described previously (Post *et al.*, 2004). To liberate LPL from endothelium, 4-h fasted mice were injected ip with heparin (0.5 U/g bodyweight; Leo Pharmaceutical Products BV, Weesp, The Netherlands) and blood was collected after 20 min; 10 µl of postheparin plasma was incubated with 0.2 ml of TG substrate mixture containing triolein (4.6 mg/ml) and [^{3}H]TO (2.5 µCi/ml) for 30 min at 37°C in the presence or absence of 1M NaCl, which completely inhibits LPL activity, to estimate both the HL and LPL activity. The LPL activity was calculated as the fraction of total triacylglycerol hydrolase activity that was inhibited by the presence of 1M NaCl and is expressed as the amount of free FA released per hour per ml of plasma.

Hepatic VLDL-TG and VLDL-apoB production. Mice were fasted for 4 h prior to the start of the experiment. During the experiment, mice were sedated as described above. At $t = 0$ min, blood was taken via tail bleeding and mice were iv-injected with 100 µl PBS containing 100 µCi Trans^{35}S-label (ICM Biomedicals, Irvine, CA) to measure *de novo* total apoB synthesis. After 30 min, the animals received 500 mg of tyloxapol (Triton WR-1339;

TABLE 1
Mean Serum Concentrations of PFAS

Compound	% in Diet (μmoles/kg)[a]	Serum concentration (mean ± SD); μg/ml (μM)[b] Experiment 1 (6 weeks, $n = 8$)	Experiment 2 (4 weeks, $n = 6$)	Experiment 3 (4 weeks, $n = 6$)
PFBS	0.03 (1003)[a]	36.7 ± 7.4^{A} $(123 \pm 25)^{b}$	37.8 ± 6.6^{A} (126 ± 22)	32.7 ± 10.2^{A} (109 ± 34)
PFHxS	0.006 (150)	217.6 ± 13.3^{A} (545 ± 33)	$197.3 \pm 10.4^{A,B}$ (494 ± 26)	188.3 ± 31.5^{B} (472 ± 72)
PFOS	0.003 (60)	124.7 ± 8.1^{A} (250 ± 16)	85.6 ± 9.5^{B} (172 ± 19)	95.3 ± 4.2^{B} (191 ± 9)

Note. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS during the indicated time periods, and serum concentrations of PFBS, PFHxS, and PFOS were measured. Values that share the same capital letter designation within a row of data are not statistically significantly different ($p > 0.05$) by the Tukey-Kramer honestly significant difference test.

[a]Dietary concentrations in parentheses are in units of μmoles/kg of diet.

[b]Fluorochemical serum concentrations in parentheses are in μM. In mice fed a control diet, serum concentrations were <0.3μM (PFBS), <0.3μM (PFHxS), and <0.2μM (PFOS).

Sigma-Aldrich) per kilogram body weight as a 10% (wt/wt) solution in sterile saline, to prevent systemic lipolysis of newly secreted hepatic VLDL-TG (Aalto-Setala *et al.*, 1992). Additional blood samples were taken at $t = 15$, 30, 60, and 90 min after tyloxapol injection and used for determination of plasma TG concentration. After 90 min, the animals were sacrificed and blood was collected by orbital puncture for isolation of VLDL by density-gradient ultracentrifugation. ^{35}S-apoB was measured in the VLDL fraction after apoB-specific precipitation with isopropanol (Egusa *et al.*, 1983; Li *et al.*, 1996; Pietzsch *et al.*, 1995).

Hepatic lipid analysis. Livers were isolated and partly homogenized (30 s at 5000 rpm) in saline (~10% wet wt/vol) using a mini-bead beater (Biospec Products, Inc., Bartlesville, OK). Lipids were extracted as described (Post *et al.*, 2000) and separated by high-performance thin-layer chromatography. Lipid spots were stained with color reagent (5 g $MnCl_2.4H_2O$, 32 ml 95–97% H_2SO_4 added to 960 ml of CH_3OH:H_2O 1:1 vol/vol) and quantified using TINA version 2.09 software (Raytest, Straubenhardt, Germany).

Fecal excretion of bile acids and neutral sterols. Fecal secretion of neutral sterols and bile acids was determined in feces, collected during a 48- to –72-h time period at two consecutive time points, by gas chromatographic analysis as described previously (Post *et al.*, 2003).

In vivo *clearance of autologous HDL.* One mouse of each experimental group was used to obtain autologous HDL that was radiolabeled with [^{3}H]CO as described (van der Hoogt *et al.*, 2007). Mice were injected via the tail vein with a trace of autologous radiolabeled HDL (0.1 μCi in 200 μl PBS). At the indicated time points after injection, blood was collected to determine the plasma decay of [^{3}H]CO. The fractional catabolic rate was calculated after curve fitting. Taking into account that plasma levels of HDL-C were changed upon treatment, the fractional catabolic rate (FCR) was also calculated from these data as millimolar HDL-C cleared per hour, based on the actual level of HDL-C in the various groups.

Hepatic gene expression analysis. Total RNA was extracted from individual livers using RNA-Bee (Bio-Connect, Huissen, The Netherlands) and glass beads according to the manufacturer's instructions. The RNA was further purified using the nucleospin RNA II kit (Machery-Nagel, Düren, Germany) according to the manufacturer's instructions. The integrity of each RNA sample obtained was examined by Agilent Lab-on-a-chip technology using an RNA 6000 Nano LabChip kit and a Bioanalyzer 2100 (Agilent Technologies, Amstelveen, The Netherlands). The Affymetrix 3′ IVT-Express labeling Kit (#901229) and the protocols optimized by Affymetrix were used to synthesize Biotin-labeled coding ribonucleic acid (cRNA) (from 100 ng of total RNA) for microarray hybridization. For the hybridization, 15 μg cRNA was used for further fragmentation and finally 10 μg for the hybridizations. The quality of intermediate products (i.e., biotin-labeled cRNA and fragmented cRNA) was again checked. Microarray analysis was carried out using an Affymetrix technology platform and Affymetrix GeneChip mouse genome 430 2.0 arrays.

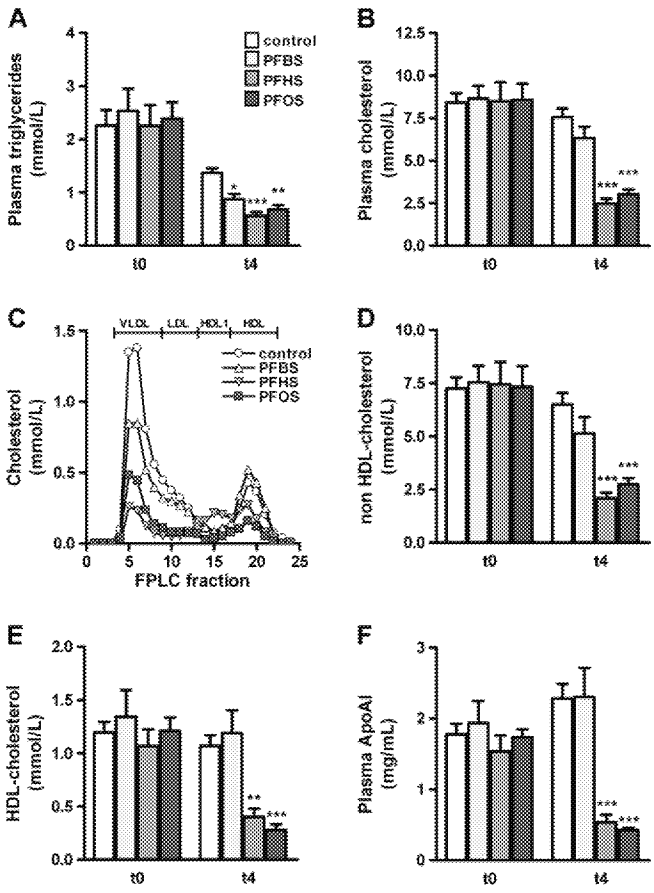

FIG. 1. Effect of PFAS on plasma TG, TC, HDL-C, and ApoAI. Mice received a Western-type diet without and with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. At baseline (t0) and after 4 weeks of intervention (t4), 4-h fasted blood was taken and plasma was assayed for TG (A) and TC (B). After 4 weeks of intervention, cholesterol distribution over lipoproteins was determined (C). Plasma at t0 and t4 were also assayed for non-HDL-C (D), HDL-C (E), and apoAI (F). Data are means ± SEM ($n = 6$). *$p < 0.01$, **$p < 0.001$, and ***$p < 0.0001$ as compared with the control group.

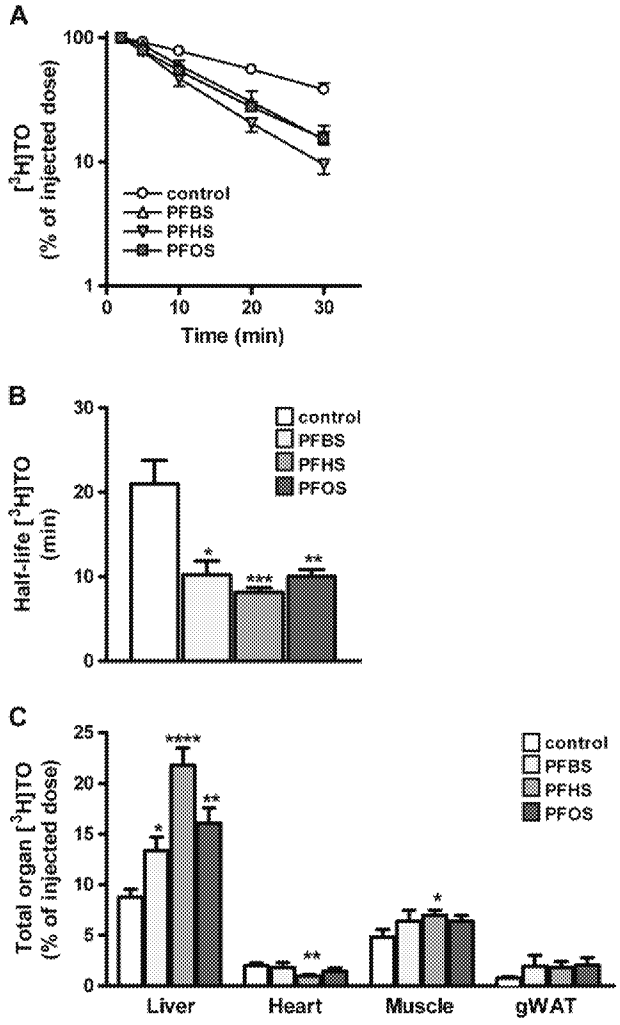

FIG. 2. Effect of PFAS on the clearance of VLDL-like emulsion particles. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. After 4-h fasting, mice were injected with VLDL-like [^{3}H]TO-labeled VLDL-like emulsion particles (1 mg TG) and plasma samples were taken at indicated time points to determine the plasma clearance of [^{3}H]TO (A). From the slopes of the curves, the half-lives of ^{3}H-activity were calculated (B). At 30 min after injection, various organs (liver, heart, skeletal muscle, and gonadal white adipose tissue [gWAT]) were harvested to determine the uptake of ^{3}H-activity (C). Data are means ± SEM (n = 4–6). *p < 0.05, **p < 0.01, ***p < 0.001, and ****p < 0.0001 as compared with the control group.

Briefly, fragmented cRNA was mixed with spiked controls and hybridized with murine GeneChip 430 2.0 arrays. The hybridization, probe array washing and staining, and washing procedures were executed as described in the Affymetrix protocols, and probe arrays were scanned with a Hewlett-Packard Gene Array Scanner (ServiceXS, Leiden, The Netherlands). Quality control of microarray data was performed using BioConductor packages (including simpleaffy and affyplm), through the NuGO pipeline that is available as a Genepattern procedure on http://nbx2.nugo.org (de Groot *et al.*, 2008). All samples passed the QC. Raw signal intensities (from CEL-files) were normalized using the GCRMA algorithm (gc-rma slow). For annotation of probes and summarization of signals from probes representing one gene the custom MNBI CDF-file was used (based on EntrezGene, version 11.0.2; http://brainarray.mbni.med.umich.edu/Brainarray/Database/CustomCDF/cdfreadme.htm). This resulted in expression values for

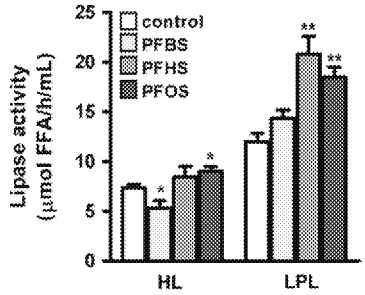

FIG. 3. Effect of PFAS on plasma HL and LPL activity. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. After 4-h fasting, heparin was injected and postheparin plasma was collected. Plasma was incubated with a [^{3}H]TO-containing substrate mixture in the absence or presence of 1M NaCl, to estimate both the HL and LPL activity. Data are means ± SEM (n = 8). *p < 0.05 and **p < 0.001 as compared with the control group.

16,331 genes, represented by unique Entrez gene identifiers. Genes were filtered for expression above five in three or more samples, resulting in a set of 11,587 genes that was used for further analysis. Gene expression data were log-transformed (base 2). Statistical analysis on resulting data was performed using the moderated t-test (Limma: http://bioinf.wehi.edu.au/limma/) with correction for multiple testing (Storey and Tibshirani, 2003). Cutoff for statistically significant changes was set at corrected p value (q value) < 0.05. In addition, T-profiler analysis (Boorsma *et al.*, 2005) was performed using expression values corrected for mean expression in the control group. This analysis resulted in scores (t-scores) and significance values for functional gene sets and biological processes (based on gene ontology annotation). Gene sets and biological processes with significant scores (> 4 or < −4) in five or six animals per group were selected.

A hierarchical clustering of these pathways and biological processes and their scores in all samples was generated in GenePattern (Broad Institute, MIT; Reich *et al.*, 2006). The data discussed in this publication have been deposited in National Center for Biotechnology Information's Gene Expression Omnibus according to MIAME compliance (GEO series accession number GSE22940).

Statistical analysis. All data are presented as means ± SEM unless indicated otherwise. Most data were analyzed using SPSS. A Kruskall-Wallis test for several independent samples was used, followed by a Mann-Whitney test for independent samples. p Values less than 0.05 were considered statistically significant. Serum PFBS, PFHxS, and PFOS data were analyzed using the Tukey-Kramer honestly significant difference test for multiple comparisons of means in JMP 5.1 (Cary, NC).

RESULTS

Serum Concentrations of PFBS, PFHxS, and PFOS

Serum concentrations of PFAS for the various experiments performed to measure all biochemical and physiological parameters are presented in Table 1. Mean serum concentrations after 4–6 weeks of feeding were 33–38 μg/ml (PFBS), 188–218 μg/ml (PFHxS), and 86–125 μg/ml (PFOS). Serum concentrations of PFBS measured in the three experiments were not different from each other. Even with much higher daily intakes of PFBS than either PFHxS or PFOS, the mean PFBS concentrations, on a molar basis, were approximately 25% those of PFHxS and 50–75% those of PFOS and serum PFHxS concentrations were approximately two to three times

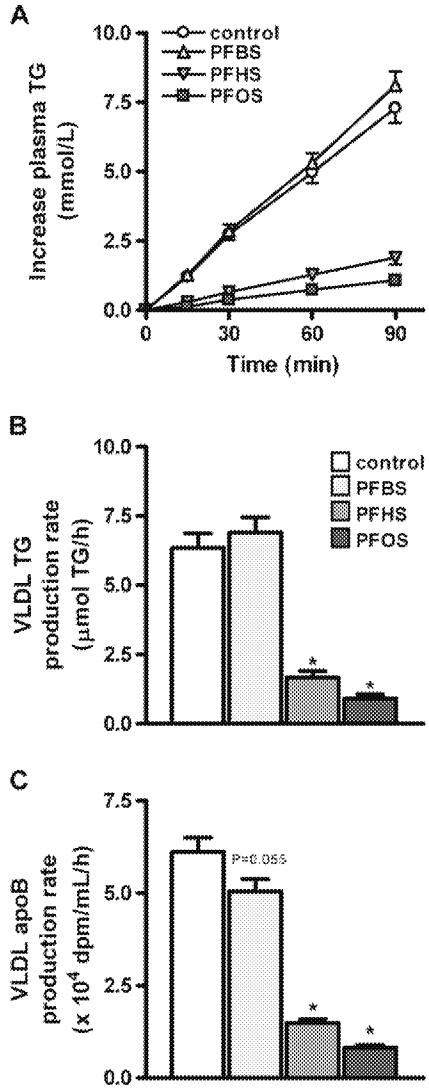

FIG. 4. Effect of PFAS on hepatic VLDL production. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. After 4-h fasting, mice were consecutively injected with Trans^{35}S-label and tyloxapol and blood samples were drawn up to 90 min after tyloxapol injection. Plasma TG concentrations were determined and plotted as the increase in plasma TG relative to $t = 0$ (A). The rate of TG production was calculated from the slopes of the curves from the individual mice (B). After 120 min, the total VLDL fraction was isolated by ultracentrifugation, and the rate of newly synthesized VLDL-apoB was determined (C). Data are means ± SEM ($n = 7$–8). *$p < 0.0001$ as compared with the control group.

those of PFOS, corresponding to the difference in daily intakes of PFHxS as compared with PFOS.

PFBS, PFHxS, and PFOS Decrease Plasma TG Levels

To investigate the effect of PFAS on lipoprotein metabolism in E3L.CETP mice, mice were fed a Western-type diet for 4 weeks. Mice were randomized (t0) and fed the same diet without or with different PFAS (0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS) for another 4 weeks (t4). Body weight and food intake did not differ between groups throughout the intervention period (data not shown). At both t0 and t4, plasma was assayed for lipids (Fig. 1). As compared with the control group, plasma TG levels were decreased by PFBS (−37%, $p < 0.01$), PFHxS (−59%, $p < 0.0001$), and PFOS (−50%, $p < 0.001$; Fig. 1A).

PFHxS and PFOS Decrease Plasma VLDL-C and HDL-C Levels

Besides TG metabolism, PFAS also affected cholesterol metabolism. Plasma TC levels were not decreased by PFBS but were significantly decreased by PFHxS (−67%, $p < 0.0001$) and PFOS (−60%, $p < 0.0001$; Fig. 1B). The tendency toward reduction of TC by PFBS was caused by a selective reduction in VLDL-C, whereas PFHxS and PFOS reduced both VLDL-C and HDL-C (Fig. 1C). Quantitative analysis showed that non-HDL-C was decreased by PFBS (−28%, $p = 0.065$), PFHxS (−68%, $p < 0.0001$), and PFOS (−60%, $p < 0.0001$; Fig. 1D). In addition, HDL-C was only decreased by PFHxS (−62%, $p < 0.001$) and PFOS (−74%, $p < 0.0001$; Fig. 1E), which was accompanied by even larger reductions in plasma apoAI induced by PFHxS (−76%, $p < 0.0001$) and PFOS (−81%, $p < 0.0001$; Fig. 1F).

PFBS, PFHxS, and PFOS Increase VLDL–TG Clearance

Plasma VLDL-TG levels are determined by the balance between VLDL-TG production and VLDL-TG clearance. To evaluate whether an increased VLDL-TG clearance may have attributed to the reduction in VLDL-TG levels caused by all PFAS, the plasma clearance of [^{3}H]TO-labeled VLDL-like emulsion particles was determined (Fig. 2). As compared with control mice, the plasma half-life of [^{3}H]TO was reduced by PFBS (−51%, $p < 0.05$), PFHxS (−61%, $p < 0.001$), and PFOS (−52%, $p < 0.01$; Figs. 2A and 2B), reflected by a significant increase in the uptake of [^{3}H]TO-derived activity by the liver and trends toward an increased uptake by skeletal muscle and white adipose tissue (WAT, Fig. 2C). Because these data are consistent with an increased lipolytic processing, HL and LPL activity were determined in postheparin plasma (Fig. 3). HL activity was decreased to some extent by PFBS (−28%, $p < 0.05$), not affected by PFHxS and increased by PFOS (+ 22%, $p < 0.05$). LPL activity was not affected by PFBS, but markedly increased by PFHxS (+ 74%, $p < 0.001$) and PFOS (+ 54%, $p < 0.001$).

PFHxS and PFOS Decrease VLDL Production

We next determined whether a reduction in VLDL-TG production may also have contributed to the TG-lowering effect of PFAS (Fig. 4). PFBS did not affect VLDL-TG production (Figs. 4A and 4B) and modestly reduced VLDL-apoB production (−17%, $p = 0.055$; Fig. 4C). In contrast, the VLDL-TG production rate was markedly decreased by PFHxS (−74%, $p < 0.0001$) and PFOS (−86%, $p < 0.0001$; Figs. 4A and 4B). The VLDL-apoB production rate was decreased to similar extents by PFHxS (−76%, $p < 0.0001$) and PFOS (−87%, $p < 0.0001$;

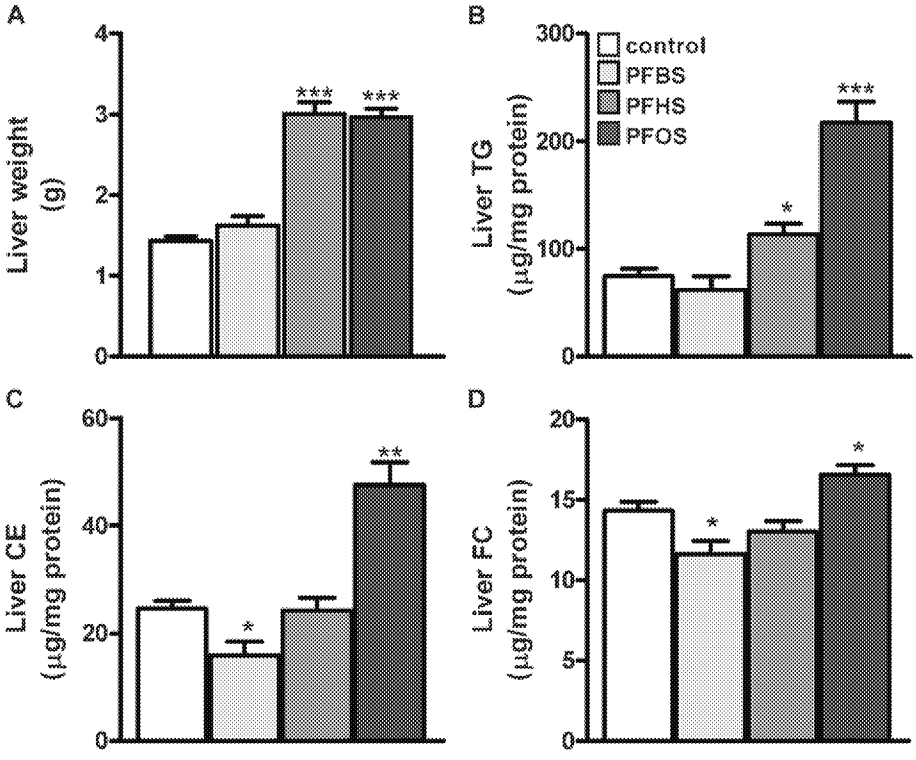

FIG. 5. Effect of PFAS on liver weight and lipid content. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. After 6 weeks, livers were collected after a 4-h fast and their weight was determined (A). Liver lipids were extracted and TG (B), CE (C), and FC (D) were quantified. Data are means ± SEM ($n = 6$). *$p < 0.05$; **$p < 0.001$; ***$p < 0.0001$ as compared with the control group.

Fig. 4C), indicating that PFHxS and PFOS both reduce the production of VLDL particles without altering their TG content.

PFHxS and PFOS Increase Liver Weight and Hepatic TG Content

To get further insight into the mechanism how PFHxS and PFOS decrease hepatic VLDL-TG production, we determined the weight and lipid content of the liver (Fig. 5). The liver weight was markedly increased by PFHxS (+ 110%, $p < 0.0001$) and PFOS (+ 107%, $p < 0.0001$; Fig. 5A), accompanied by an increased hepatic TG content (+ 52%, $p < 0.05$, and + 192%, $p < 0.0001$, respectively; Fig. 5B). PFOS also markedly increased hepatic cholesteryl esters (CE; + 94%, $p < 0.001$; Fig. 5C) and mildly increased free cholesterol (FC; + 16%, $p < 0.05$; Fig. 5D). PFBS on the other hand decreased both hepatic CE (−36%, $p < 0.05$) and FC (−19%, $p < 0.05$; Figs. 5C and 5D). These data indicate that PFHxS and PFOS decrease VLDL-TG production as a result of impaired TG secretion from the liver, leading to hepatomegaly and hepatic steatosis, rather than being a consequence of reduced hepatic lipids available for VLDL production.

PFHxS and PFOS Decrease White Perigonadal Fat Pad Weight, Plasma-Free FAs and Plasma Glycerol

Because the accumulation of hepatic TG as induced by PFHxS and PFOS, accompanied by a marked reduction in VLDL-TG production, may result in reduced supply of VLDL-TG–derived FA for storage in adipose tissue, we determined the effect of PFAS on perigonadal fat pad weight (Fig. 6A). Indeed, perigonadal fat pad weight was decreased by PFHxS (−28%, $p < 0.01$) and PFOS (−25%, $p < 0.05$), whereas PFBS had no effect. This was accompanied by reduced plasma FA (−41%, $p < 0.0001$, and −37%, $p < 0.0001$; Fig. 6B) and plasma glycerol (−50%, $p < 0.0001$, and −42%, $p < 0.0001$; Fig. 6C), both of which are mainly derived from TG lipolysis in adipose tissue.

PFHxS and PFOS Decrease Fecal Bile Acid Excretion

To determine whether the changes in hepatic lipid content were accompanied by an effect on cholesterol excretion into feces, the effect of the PFAS on fecal output of neutral sterols and bile acids was determined (Fig. 7). The various PFAS did not affect neutral sterol secretion (Fig. 7A), but excretion of bile acids was decreased by PFHxS (−41%, $p < 0.05$) and PFOS (−50%, $p < 0.01$; Fig. 7B).

PFHxS and PFOS Decrease HDL-C Clearance

Because both PFHxS and PFOS decreased plasma levels of HDL-C and apoAI, we investigated whether this was caused by increased HDL turnover. HDL-C clearance was determined using autologous [^{3}H]CO-labeled HDL (Fig. 8). Treatment with PFHxS and PFOS increased the clearance of the [^{3}H]CO tracer (Fig. 8A), reflected by a decrease of the half-life of [^{3}H]CO

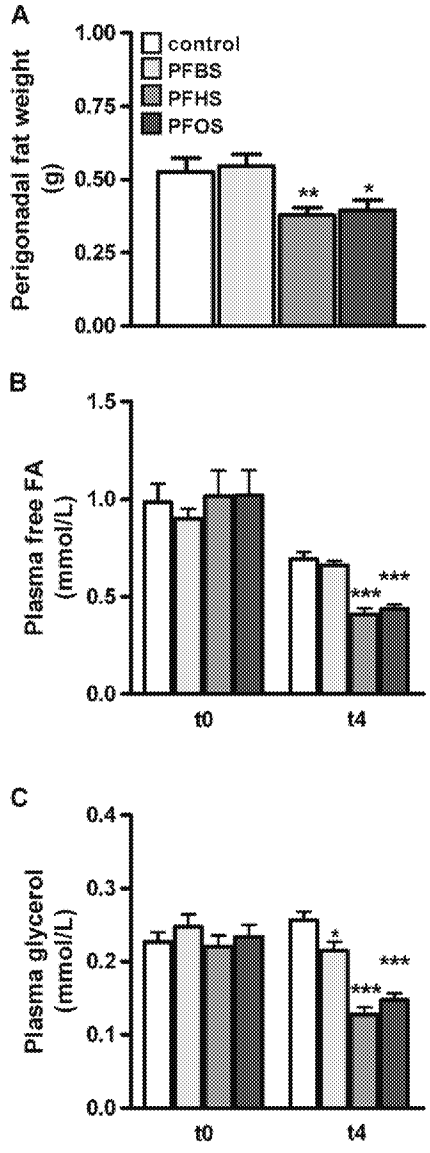

FIG. 6. Effect of PFAS on perigonadal fat pad weight, plasma FA and plasma glycerol. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. After 4 weeks, the perigonadal fat pads were collected and their weights were measured (A). At baseline (t0) and after 4 weeks of intervention (t4), blood samples were taken after a 4-h fast and plasma was assayed for FA (B) and glycerol (C). Data are means ± SEM ($n = 6$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.0001$ as compared with the control group.

(PFHxS −33%, $p < 0.001$; PFOS −35%, $p < 0.0001$; Table 2). However, calculation of the FCR of HDL-C, taking into account the different pool size of HDL-C after treatment with the various PFAS, showed that the clearance of HDL-C (calculated as millimolar HDL-C cleared per hour) was actually decreased by PFHxS (−48%, $p < 0.01$) and PFOS (−65%, $p < 0.001$). The reduction in HDL-C clearance may be related to a decrease in plasma CETP activity. Indeed, plasma CETP mass was decreased by PFBS (−20%, $p < 0.01$), PFHxS (−36%, $p < 0.001$), and PFOS (−38%, $p < 0.0001$; Fig. 8B). Collectively, these data indicate that the observed reduction in plasma HDL-C is likely caused by impaired production and/or maturation of HDL particles rather than enhanced clearance.

PFHxS and PFOS Affect Hepatic Expression of Genes Involved in Lipid Metabolism

To further investigate the mechanisms by which PFAS affect lipid metabolism, we determined the hepatic expression profile of 16,331 well-characterized mouse genes. As compared with the control group, PFAS resulted in 438 (PFBS), 4230 (PFHxS), and 3986 (PFOS) differentially expressed genes (Fig. 9). A selection of genes involved in lipid metabolism is depicted in Table 3. In general, the expression of many of these genes was affected by PFHxS and PFOS, but not by PFBS.

Both PFHxS and PFOS affected genes involved in VLDL metabolism. PFHxS and PFOS largely increased *Lpl* expression. Although it should be noted that LPL expression in liver is low as compared with heart, muscle, and WAT, this is in line with increased VLDL-TG clearance and plasma LPL activity. Despite some differences with respect to the individual effects of both compounds, PFHxS and PFOS both upregulated genes involved in FA uptake and transport (*Slc27a1*, *Slc27a2*, *Slc27a4*, and *Cd36*), FA binding and activation (*Fabp4*, *Acsl1*, *Acsl3*, and *Acsl4*), and FA oxidation (*Cpt1b*, *Acox1*, *Acox2*, *Ehhadh*, *Acaa1a*, and *Acaa1b*). PFHxS and PFOS also increased important genes involved in TG synthesis and VLDL assembly/secretion (*Dgat1*, *Scd2*, and *Mttp*). Taken together, the liver increases FA oxidation, binding and activation, and mobilizes FA for TG synthesis and secretion as VLDL. Most likely, these pathways are overshadowed by increased FA uptake and transport resulting in hepatomegaly with hepatic TG accumulation, possibly related to pregnane X receptor (PXR) activation (Moreau *et al.*, 2008). PFHxS and PFOS also affected genes involved in HDL metabolism. They decreased genes involved in HDL synthesis (*Apoa1*) and maturation (*Abca1* and *Lcat*) and decreased the principle gene involved in HDL clearance (*Scarb1*). These data suggest that the observed large reduction in both HDL-C and apoAI may be caused by decreased HDL synthesis and maturation, which may result in a compensatory increase in SR-BI expression. Finally, PFHxS and PFOS affected genes in hepatic cholesterol metabolism. Both increased *Acat1*, involved in storage of cholesterol as CE, and decreased genes involved in bile acid formation (*Cyp7a1*) and secretion (*Slc10a1*, *Slc10a2*, and *Abcb11*) as well as cholesterol excretion (*Abcg5* and *Abcg8*). These data are in line with the observation that PFOS increases the hepatic cholesterol content and that both PFHS and PFOS decrease fecal bile acid secretion.

DISCUSSION

This study investigated the mechanism underlying the effect of PFHxS and PFOS on plasma lipoprotein metabolism, as well as the importance of the alkyl chain length using E3L.CETP mice,

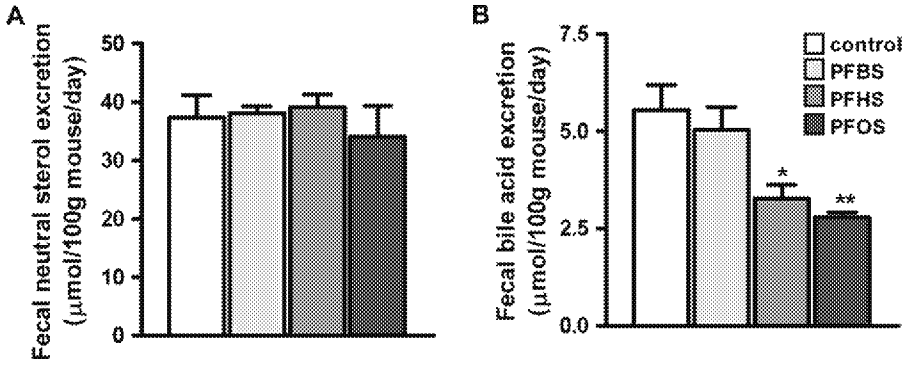

FIG. 7. Effect of PFAS on fecal bile acid excretion. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. Feces were collected during a 48- to 72-h time period at two consecutive time points, and neutral sterols (A) and bile acids (B) were quantified by gas chromatographic analysis. Data are means ± SEM ($n = 6$). *$p < 0.05$; **$p < 0.01$ as compared with the control group.

a well-established model for human-like lipoprotein metabolism (Bijland *et al.*, 2010; de Haan *et al.*, 2008a,b; van der Hoogt *et al.*, 2007, 2008). At high exposure levels, PFHxS and PFOS lowered plasma TG, VLDL-C, and HDL-C, which is consistent with previous observations in toxicological studies (Berthiaume and Wallace, 2002; Curran *et al.*, 2008; Haughom and Spydevold, 1992; Manal *et al.*, 2009; Martin *et al.*, 2007; Seacat *et al.*, 2002, 2003). Under the study conditions and considering the molar concentrations given in diet, PFOS was more effective than PFHxS, followed by PFBS, in lowering plasma lipids. This may be, in part, due to pharmacokinetic differences; however, the molar serum PFHxS concentrations achieved were more than twice those achieved with PFOS. In addition, Bjork and Wallace (Bjork and Wallace, 2009) have demonstrated molecular weight–dependent increasing potency of PFBS, PFHxS, and PFOS to induce expression of *Cte/acot1*, *Cyp4A1/Cyp4A11*, and *Acox* in rat and human primary hepatocytes at a concentration of 25μM *in vitro*. Mechanistic studies revealed that PFHxS and PFOS decreased lipoprotein levels primarily by severely impairing the production of VLDL and HDL, resulting in hepatomegaly with steatosis as well as combined hypolipidemia.

VLDL levels were decreased by all PFAS tested, albeit with different potency. Considering that the daily intake of PFBS (30 mg/kg/day) was five times that of PFHxS (6 mg/kg/day) and 10 times that of PFOS (3 mg/kg/day), PFBS had considerably less effects compared with PFHxS and PFOS. This may, in part, be due to the much lower serum PFBS concentrations than those observed for PFHxS and PFOS. All PFAS tested accelerated VLDL-TG clearance from plasma to a similar extent. PFHxS and PFOS increased plasma LPL activity as well as LPL messenger RNA (mRNA) in the liver, suggesting that LPL activity is increased due to an overall higher LPL expression, which increases the capacity of plasma to enhance VLDL-TG clearance. Accordingly, the uptake of FA was increased in the LPL-expressing organs, skeletal muscle, and WAT, as well as in the liver (i.e., mainly within VLDL remnants). In contrast, PFBS did not increase hepatic LPL mRNA or plasma LPL activity, nor did it differentially affect hepatic gene expression of activators (apoAV and apoCII) or inhibitors (apoCI and apoCIII) of LPL activity as compared with PFHxS and PFOS. This suggests that PFBS accelerates VLDL-TG clearance through a different, as yet unidentified mechanism.

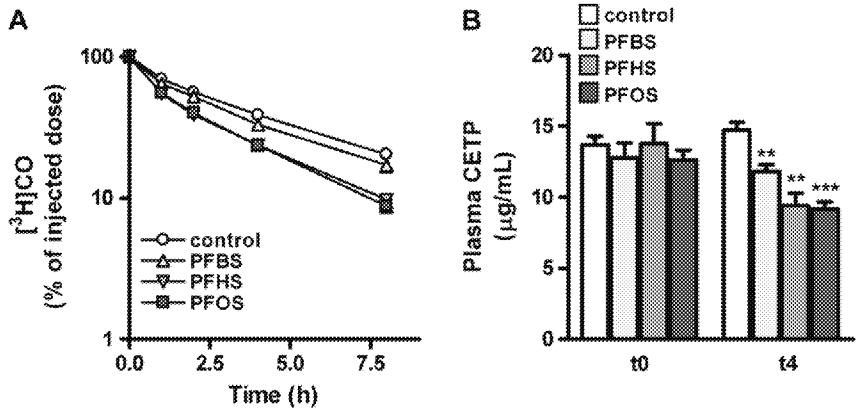

FIG. 8. Effect of PFAS on the clearance of HDL-C. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. Mice were injected with autologous [^{3}H]CO-labeled HDL, and plasma samples were taken at indicated time points to determine the plasma clearance of [^{3}H]CO (A). In a separate experiment, 4-h fasted blood was taken at baseline (t0) and after 4 weeks of intervention (t4) and plasma was assayed for CETP mass (B). Data are means ± SEM ($n = 5$). *$p < 0.01$; **$p < 0.001$; ***$p < 0.0001$ as compared with control.

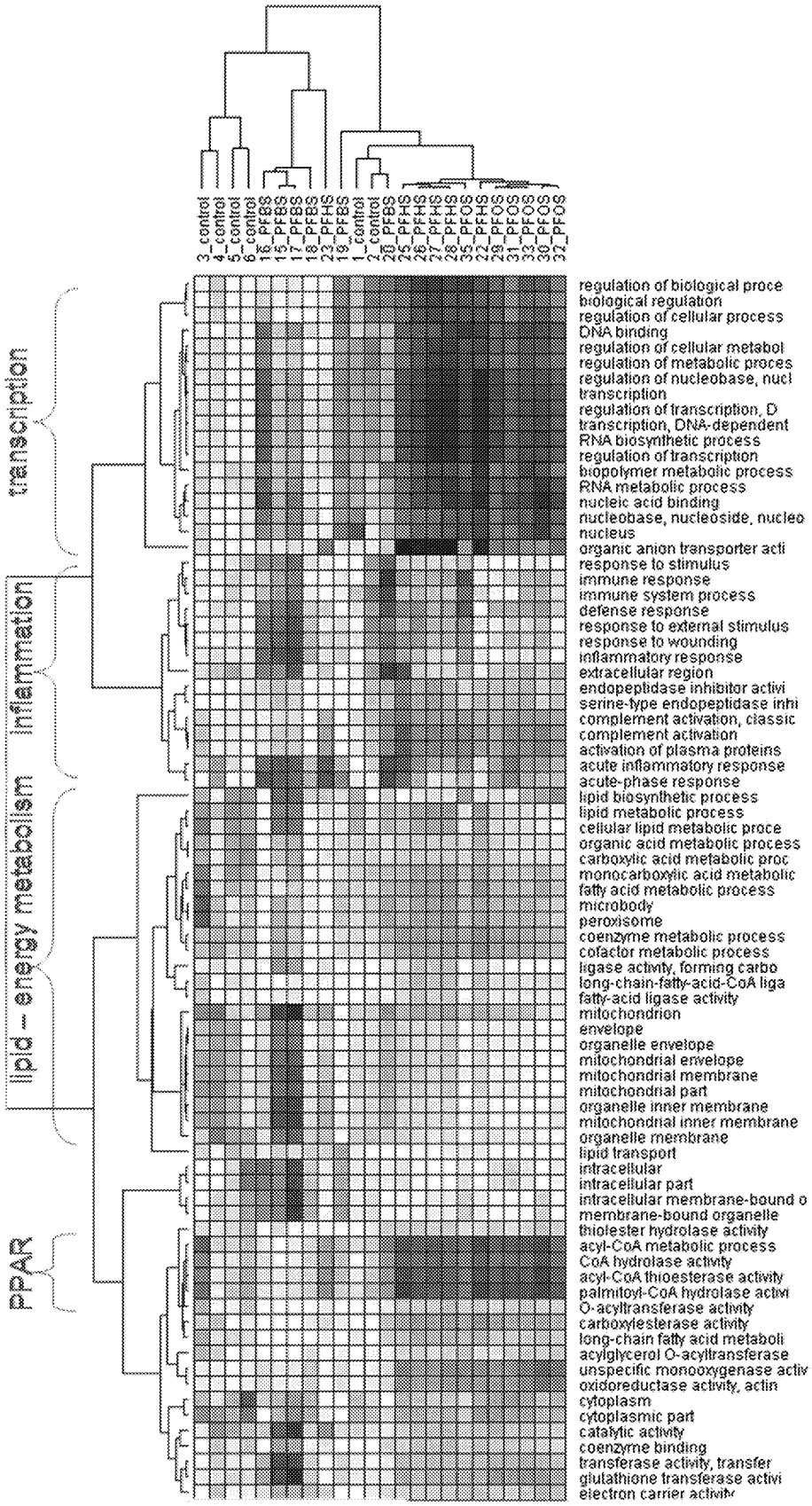

TABLE 2
Effect of PFAS on the Fractional Catabolic Rate of HDL-C

	Control	PFBS	PFHxS	PFOS
$T_{½}$ (h)	3.26 ± 0.11	2.89 ± 0.15	2.19 ± 0.12**	2.13 ± 0.09***
FCR (pools HDL-C/h)	0.21 ± 0.01	0.24 ± 0.01	0.32 ± 0.02**	0.33 ± 0.01***
FCR (mM HDL-C/h)	0.23 ± 0.02	0.25 ± 0.04	0.12 ± 0.03 **	0.08 ± 0.02**

Note. Mice received a Western-type diet without or with 0.03% PFBS, 0.006% PFHxS, or 0.003% PFOS for 4 weeks. Mice were injected with autologous [^{3}H]CO-labeled HDL. The data from Supplementary Figure 3A were used to calculate the plasma half-life and FCR as pools or millimolar of HDL-C cleared per hour. Values are means ± SEM ($n = 6$).

*$p < 0.01$, **$p < 0.001$, and ***$p < 0.0001$.

The observation that PFHxS and PFOS were more effective in VLDL lowering than PFBS can be explained by the fact that PFHxS and PFOS, but not PFBS, severely impaired hepatic VLDL-TG production by as much as ~80%. This was a result of reduced VLDL particle production because PFHxS and PFOS equally decreased the production of VLDL-TG and VLDL-apoB. The decreased VLDL-TG production rate is presumably not explained by reduced availability of liver TG for VLDL synthesis because both PFHxS and PFOS increased rather than reduced the hepatic TG content, similarly as observed in rats (Haughom and Spydevold, 1992). More likely, PFHxS and PFOS prevent the secretion of VLDL from the liver, resulting in lipid accumulation within the liver. This increase in liver lipid content was accompanied by increased expression of hepatic genes involved in FA binding and activation and FA β-oxidation, probably resulting from Peroxisome proliferator-activated receptor (PPAR)α activation, and by increased expression of genes involved in mobilization of FA for TG synthesis (*Dgat1*) and secretion as VLDL (*Mttp*). Interestingly, the VLDL production-lowering effect of PFHxS and PFOS is accompanied by decreased fecal bile acid excretion coupled with decreased hepatic expression of *Cyp7a1*. Decreased expression of *Cyp7a1* may actually contribute to the reduction in VLDL production, as we previously observed that *Cyp7a1* deficiency in E3L mice decreased VLDL-TG production (Post *et al.*, 2004), and *Cyp7a1*-transgenic mice show increased VLDL production (Miyake *et al.*, 2001).

Collectively, the data suggest that PFHxS and PFOS primarily impair the secretion of VLDL by the liver, leading to hepatic steatosis. The reduced production of VLDL-TG limits substrate availability for LPL on peripheral tissues, leading to less FA delivery to WAT and skeletal muscle. Because LPL-mediated delivery of VLDL-TG–derived FA is a strong determinant of WAT mass and obesity (Voshol *et al.*, 2009), this can explain why PFHxS and PFOS, but not PFBS, reduced gonadal WAT mass accompanied by a reduction of plasma-free FA and glycerol that are mainly derived from TG lipolysis in adipose tissue.

PFHxS and PFOS also markedly decreased HDL levels, reflected by a reduction in HDL-C and apoAI. Because PFHxS and PFOS decreased HDL turnover, in terms of millimolar HDL-C cleared per hour, these compounds thus lower HDL levels by reducing the synthesis and maturation of HDL. This is further supported by the fact that PFHxS and PFOS, but not PFBS, decrease the hepatic expression of *Apoa1*, *Abca1*, and *Lcat*, important for the generation of discoidal HDL precursors (apoAI), lipidation of these HDL precursors (ABCA1), and maturation of HDL (LCAT). The mechanism underlying the decrease in HDL turnover may relate to the observed decrease in hepatic *Scarb1* expression and plasma CETP mass.

Nuclear receptors are important regulators of lipid metabolism and changes in their expression patterns might thus underlie the effects of PFHxS and PFOS on lipid metabolism. PFOS has been demonstrated to transactivate PPARα (Shipley *et al.*, 2004) and most effects of PFAA have, in fact, been attributed to the activation of PPARα pathway (Bjork *et al.*, 2008; Curran *et al.*, 2008; Haughom and Spydevold, 1992; Ikeda *et al.*, 1985). Indeed, PFHxS and PFOS increased the hepatic expression of genes involved in both FA uptake and FA β-oxidation and induced hepatomegaly, similar as observed after treatment of E3L.CETP mice with the PPARα agonist fenofibrate (Bijland *et al.*, 2010). However, PFHxS and PFOS also caused hepatic steatosis, decreased VLDL-TG production, and decreased HDL levels, whereas fenofibrate did not induce hepatic steatosis, increased VLDL-TG production, and increased HDL levels (Bijland *et al.*, 2010), indicating that additional pathways must be involved.

Xenobiotic metabolism that is provoked by chemical pollutants for detoxification likely represents such an additional pathway. This involves the xenosensor receptors, PXR, and constitutive androstane receptor (CAR). Initially identified as xenosensors, it is now evident that PXR (and CAR) also trigger pleiotropic effects on liver function (Moreau *et al.*, 2008). PFOS has been shown to interact with PXR and CAR (Ren *et al.*, 2009). In the present study, PFOS increased PXR expression, accompanied by an increase in *Cyp3a11* (1.2-fold) and decrease in *Cyp7a1* (−3.8-fold), both typical for PXR activation. In fact, the effect of PFOS on both liver TG and cholesterol accumulation and reduction in HDL and fecal bile acids may all be explained by PXR activation. Indeed, specific PXR activation in E3L.CETP mice also reduces HDL accompanied by decreased hepatic expression of apoAI,

←

FIG. 9. Hierarchical clustering of scores for biological processes. T-profiler analysis was performed using expression values corrected for mean expression in the control group. Pathways and biological processes with significant scores (> 4 or < −4) in five or six animals of at least one of the PFAS groups were selected. A hierarchical clustering of these pathways and biological processes and their scores in all samples was generated in GenePattern (Broad Institute). Red indicates positive score (majority of genes in set are upregulated), blue indicate negative score (majority of genes in set are down-regulated).

TABLE 3
Effect of PFAS on Hepatic Expression of Genes Encoding Transcription Factors and Proteins Involved in Lipid Metabolism

		PFBS		PFHS		PFOS	
Protein	Gene	Δ	*q* Value	Δ	*q* Value	Δ	*q* Value
Transcription factors							
LXR alpha	*Nr1h3*	−1.00	0.692	**−1.19**	**0.039**	**−1.27**	**0.008**
LXR beta	*Nr1h2*	−1.16	0.304	**−1.24**	**0.046**	−1.08	0.277
PPAR alpha	*Ppara*	1.10	0.453	**−1.40**	**0.002**	**−1.40**	**0.003**
PPAR gamma	*Pparg*	1.07	0.619	1.25	0.179	**1.52**	**0.042**
CAR	*Nr1i3*	−1.13	0.546	−1.01	0.443	**1.82**	**0.007**
FXR	*Nr1h4*	−1.01	0.676	**1.19**	**0.035**	**1.21**	**0.028**
PXR	*Nr1i2*	−1.03	0.622	**1.28**	**0.006**	**1.59**	**<0.001**
PGC1alpha	*Ppargc1a*	−0.18	0.547	**−1.39**	**<0.001**	**−1.27**	**<0.001**
PGC1beta	*Ppargc1b*	−0.30	0.458	−0.47	0.119	−0.44	0.139
Lipolysis							
LPL	*Lpl*	1.02	0.666	**4.27**	**<0.001**	**2.13**	**<0.001**
ApoCI	*Apoc1*	−1.04	0.285	−1.03	0.209	**−1.07**	**0.020**
ApoCII	*Apoc2*	1.01	0.672	−1.16	0.056	**−1.30**	**0.003**
ApoCIII	*Apoc3*	−1.00	0.683	−1.04	0.139	−1.01	0.370
ApoAV	*Apoa5*	**−1.32**	**0.013**	**−2.01**	**<0.001**	**−1.61**	**<0.001**
GPIHBP1	*Gpihbp1*	−1.25	0.189	1.19	0.103	**1.36**	**0.012**
FA uptake and transport							
FATPa1	*Slc27a1*	1.20	0.414	**2.90**	**<0.001**	**2.33**	**<0.001**
FATPa2	*Slc27a2*	1.10	0.060	**1.08**	**0.022**	**1.07**	**0.043**
FATPa4	*Slc27a4*	−1.01	0.684	**2.84**	**<0.001**	**2.08**	**<0.001**
FATPa5	*Slc27a5*	−1.00	0.683	**−1.11**	**0.047**	−1.08	0.127
CD36	*Cd36*	**1.62**	**0.001**	**3.48**	**<0.001**	**3.40**	**<0.001**
LDLR	*Ldlr*	1.02	0.672	1.14	0.234	−1.23	0.137
PCSK9	*Pcsk9*	−1.08	0.636	1.40	0.172	−1.48	0.142
FA binding and activation							
FABP1	*Fabp1*	−1.02	0.573	**1.07**	**0.048**	1.06	0.087
FABP2	*Fabp2*	−1.30	0.069	**−2.04**	**<0.001**	**−2.20**	**<0.001**
FABP4	*Fabp4*	1.51	0.077	**3.25**	**<0.001**	**2.21**	**<0.001**
FABP6	*Fabp6*	1.03	0.643	−1.12	0.199	−1.08	0.303
FABP7	*Fabp7*	−1.16	0.546	**−2.20**	**0.004**	−1.35	0.169
ACSL1	*Acsl1*	**1.31**	**0.008**	**1.85**	**<0.001**	**1.73**	**<0.001**
ACSL3	*Acsl3*	1.19	0.482	**2.98**	**<0.001**	**1.71**	**0.016**
ACSL4	*Acsl4*	1.19	0.170	**2.08**	**<0.001**	**1.40**	**0.001**
ACSL5	*Acsl5*	−1.10	0.437	**1.42**	**0.002**	1.03	0.407
ACSS1	*Acss1*	−1.01	0.671	**1.23**	**0.021**	1.18	0.057
ACSS2	*Acss2*	−1.24	0.498	**1.95**	**0.023**	−1.26	0.257
ACSM1	*Acsm1*	1.03	0.568	**−1.19**	**0.004**	−1.04	0.305
ACSM2	*Acsm2*	1.19	0.057	1.02	0.360	−1.02	0.371
ACSM3	*Acsm3*	−1.27	0.080	**−1.32**	**0.005**	**−1.40**	**0.001**
ACSM5	*Acsm5*	−1.24	0.116	−1.05	0.316	1.06	0.300
FA oxidation							
CPT1a	*Cpt1a*	1.01	0.675	−1.16	0.067	−1.12	0.143
CPT1b	*Cpt1b*	1.04	0.645	**2.91**	**<0.001**	**2.37**	**<0.001**
ACO1	*Acox1*	1.14	0.071	**1.46**	**<0.001**	**1.39**	**<0.001**
ACO2	*Acox2*	1.02	0.629	**1.20**	**0.003**	**1.33**	**<0.001**
ACO3	*Acox3*	1.07	0.506	−1.16	0.081	−1.08	0.240
Bifunctional enzyme	*Ehhadh*	**1.37**	**0.008**	**3.19**	**<0.001**	**2.89**	**<0.001**
Thiolase 1a	*Acaa1a*	1.21	0.154	**1.83**	**<0.001**	**1.82**	**<0.001**
Thiolase 1b	*Acaa1b*	1.10	0.069	**1.29**	**<0.001**	**1.24**	**<0.001**
Thiolase 2	*Acaa2*	−1.03	0.479	**1.08**	**0.029**	1.06	0.107
FA/TG synthesis							
FAS	*Fasn*	−1.42	0.403	1.53	0.135	−1.13	0.374
DGAT1	*Dgat1*	−1.05	0.581	**1.39**	**0.006**	**1.37**	**0.008**
DGAT2	*Dgat2*	−1.09	0.360	**−1.25**	**0.005**	**−1.16**	**0.042**
SCD1	*Scd1*	−1.54	0.109	1.18	0.226	−1.03	0.420
SCD2	*Scd2*	1.14	0.282	**1.65**	**<0.001**	**1.25**	**0.013**
ACLY	*Acly*	−1.49	0.292	1.11	0.366	**−2.47**	**0.003**
S14	*Thrsp*	−1.28	0.516	1.17	0.349	1.12	0.389
VLDL assembly							
ApoB	*Apob*	−1.00	0.672	−1.07	0.073	**−1.11**	**0.012**
ApoBEC	*Apobec1*	**1.50**	**0.045**	1.16	0.174	1.03	0.413
MTP	*Mttp*	1.18	0.171	**1.27**	**0.007**	**1.22**	**0.020**
Cholesterol synthesis							
ACLY	*Acly*	−1.49	0.292	1.11	0.366	**−2.47**	**0.003**
HMG CoA reductase	*Hmgcr*	−1.07	0.629	**1.62**	**0.045**	−1.28	0.204
HMG CoA synthase	*Hmgcs2*	1.03	0.573	−1.03	0.337	1.05	0.244
Squalene synthase	*Fdft1*	−1.25	0.342	**1.78**	**0.003**	−1.08	0.360
Cholesterol storage							
ACAT1	*Acat1*	−1.06	0.370	**1.17**	**0.002**	**1.10**	**0.034**
ACAT2	*Acat2*	−1.10	0.569	**1.77**	**0.006**	−1.03	0.429
Cholesterol uptake							
LDLR	*Ldlr*	1.02	0.672	1.14	0.234	−1.23	0.137
PCSK9	*Pcsk9*	−1.08	0.636	1.40	0.172	−1.48	0.142
Cholesterol metabolism							
CYP7A1	*Cyp7a1*	−1.19	0.624	**−3.20**	**0.037**	**−3.78**	**0.021**
IBAT	*Slc10a2*	1.52	0.300	**−2.45**	**0.005**	**−2.80**	**0.002**
BSEP	*Abcb11*	−1.19	0.198	**−1.65**	**<0.001**	**−1.74**	**<0.001**
NTCP	*Slc10a1*	−1.24	0.191	**−1.32**	**0.020**	**−1.34**	**0.014**
Cholesterol excretion							
ABCG5	*Abcg5*	−1.38	0.061	**−2.06**	**<0.001**	**−1.83**	**<0.001**
ABCG8	*Abcg8*	−1.61	0.061	**−2.46**	**<0.001**	**−2.38**	**<0.001**
HDL formation							
ApoAI	*Apoa1*	−1.10	0.148	**−1.35**	**<0.001**	**−1.45**	**<0.001**
ApoAII	*Apoa2*	−1.05	0.289	−1.02	0.270	−1.01	0.373
HDL maturation							
ABCA1	*Abca1*	1.01	0.635	**−1.14**	**0.024**	−1.09	0.092
LCAT	*Lcat*	1.01	0.669	**−1.43**	**<0.001**	**−1.35**	**<0.001**
HDL remodeling							
PLTP	*Pltp*	−1.07	0.629	**2.28**	**0.002**	1.31	0.171
Endothelial lipase	*Lipg*	−1.15	0.457	−1.04	0.398	−1.19	0.185
HL	*Lipc*	−1.17	0.081	**−1.70**	**<0.001**	**−1.40**	**<0.001**
HDL uptake							
SRB1	*Scarb1*	−1.20	0.168	**−2.31**	**<0.001**	**−1.76**	**<0.001**

Note. Mice received a Western-type diet without or with PFAS. Livers were collected after a 4-h fast, total RNA was extracted from livers of individual mice ($n = 6$ per group), and gene expression analysis was performed using Affymetrix GeneChip mouse genome 430 2.0 arrays. Data represent mean fold change (Δ) as compared with the control group. *q* Values are corrected for multiple testing. Values in bold are considered significant (*q* value < 0.05).

ABCA1, LCAT, and HL (de Haan *et al.*, 2009). In addition, both PCN (de Haan *et al.*, 2009) and constitutive PXR expression (Zhou *et al.*, 2006) caused accumulation of TG and cholesterol in the liver (Moreau *et al.*, 2008), which is likely explained by the profound induction of FA transport genes including CD36 and FATP as observed in this study. Collectively, our data thus suggest that the effects of PFAS on lipid metabolism are the combined result of activation of nuclear receptors that include at least PPARα and PXR with PFHxS and PFOS as most potent activators.

This study confirms the findings from previous toxicological studies in animal models that PFHxS and PFOS reduce plasma TG and TC (Berthiaume and Wallace, 2002; Curran *et al.*, 2008; Haughom and Spydevold, 1992; Manal *et al.*, 2009; Martin *et al.*, 2007; Seacat *et al.*, 2002, 2003) and provides mechanistic explanations. These observations are in seeming contrast with epidemiological studies in humans that have shown modest increases in non-HDL-C. Reductions in TC and TG have not been observed among exposed workers (Olsen *et al.*, 2003) with serum PFOS concentrations as high as 10 μg/ml. On the contrary, recent cross-sectional studies in general population cohorts from the United States even showed positive correlations between PFOS levels and serum cholesterol due to a positive correlation with non-HDL-C (Nelson *et al.*, 2010; Steenland *et al.*, 2009). However, a positive association of PFOS with non-HDL-C in workers with significantly higher serum concentrations than those found in the general population studies was not found (Olsen *et al.*, 2003). In a recent report, no association of serum PFOS was found for either LDL or non-HDL-C, whereas a positive association was found for HDL-C in 723 adult Nunavik Inuit (Chateau-Degat *et al.*, 2010). It should be noted that species differences between mice and humans with respect to the effects of PFAS on plasma lipid metabolism cannot be excluded and that the E3L.CETP mouse merely remains a model for human-like lipid metabolism. However, these observations and the mechanistic work reported in our present study do suggest that the positive correlation between serum PFOS levels and cholesterol levels that has been found in some general population cohorts is not causal.

Large species differences have been reported with respect to the relative PFOS-induced activation of PPARα, PXR, and CAR (Ren *et al.*, 2009). Because humans have ~10-fold lower expression of PPARα in liver compared with mice (Tilton *et al.*, 2008), it is possible that, in humans, PPARα effects do not manifest themselves until higher body burdens are achieved, which would be consistent with the observations in cynomolgus monkeys given daily capsule doses of PFOS (Bjork and Wallace, 2009; Seacat *et al.*, 2002). However, differences could also be related to the recent observations that PPARα regulates a mostly divergent set of genes in mouse and human hepatocytes (Rakhshandehroo *et al.*, 2009). In the study of Seacat *et al.* (2002), a marker of peroxisome proliferation was increased marginally but with statistical significance in male and female cynomolgus monkeys only in the high-dose group (0.75 mg/kg/day for 6 months). Serum TC and HDL-C were strongly reduced in this group without a reduction in TG, and hepatic steatosis was clearly apparent. Thus, these findings support the potential of a mixed PPARα and PXR response in the monkey.

In conclusion, we have demonstrated that PFHxS and PFOS reduce plasma TG and TC in E3L.CETP mice, by lowering both VLDL and HDL. Lowering of VLDL was the result of a decreased hepatic VLDL-TG production and increased VLDL-TG clearance. Lowering of HDL was explained by decreased production and maturation. These effects are dependent on the alkyl chain length as PFBS had negligible effects and can be explained by the combined action of PPARα and PXR/CAR activation.

FUNDING

Nutrigenomics Consortium/Top Institute Food and Nutrition; the Center for Medical Systems Biology; the Netherlands Consortium for Systems Biology, within the framework of the Netherlands Genomics Initiative (NGI/NWO); the Netherlands Organization for Health Care Research Medical Sciences (Zon-MW grant 948.000.04); the Netherlands Organization for Scientific Research (NWO VIDI grant 917.36.351 to P.C.N.R.); the Netherlands Heart Foundation (2009T038 to P.C.N.R., an Established Investigator).

ACKNOWLEDGMENTS

This work was performed within the framework of the Leiden Center for Cardiovascular Research LUMC-TNO. We thank Marian Bekkers, Simone van der Drift-Droog, and Karin Toet for excellent technical assistance. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. S.C.C., D.J.E., and J.L.B. are employees of 3M Company, who also provided financial support for this study. 3M Company was involved in the study design and preparation of the manuscript (S.C.C., D.J.E., and J.L.B) but had no role in data collection apart from determination of serum concentrations of PFAS or decision to publish.

REFERENCES

Aalto-Setala, K., Fisher, E. A., Chen, X., Chajek-Shaul, T., Hayek, T., Zechner, R., Walsh, A., Ramakrishnan, R., Ginsberg, H. N., and Breslow, J. L. (1992). Mechanism of hypertriglyceridemia in human apolipoprotein (apo) CIII transgenic mice. Diminished very low density lipoprotein fractional catabolic rate associated with increased apo CIII and reduced apo E on the particles. *J. Clin. Invest.* **90,** 1889–1900.

Berthiaume, J., and Wallace, K. B. (2002). Perfluorooctanoate, perfluorooctanesulfonate, and N-ethyl perfluorooctanesulfonamido ethanol; peroxisome proliferation and mitochondrial biogenesis. *Toxicol. Lett.* **129,** 23–32.

Bijland, S., Pieterman, E. J., Maas, A. C., van der Hoorn, J. W., Van Erk, M. J., Van Klinken, J. B., Havekes, L. M., Willems van, D. K., Princen, H. M., and Rensen, P. C. (2010). Fenofibrate increases very low density lipoprotein-

triglyceride production despite reducing plasma triglyceride levels in APOE*3-Leiden.CETP mice. *J. Biol. Chem.* **285,** 25168–25175.

Bjork, J. A., Lau, C., Chang, S. C., Butenhoff, J. L., and Wallace, K. B. (2008). Perfluorooctane sulfonate-induced changes in fetal rat liver gene expression. *Toxicology* **251,** 8–20.

Bjork, J. A., and Wallace, K. B. (2009). Structure-activity relationships and human relevance for perfluoroalkyl acid-induced transcriptional activation of peroxisome proliferation in liver cell cultures. *Toxicol. Sci.* **111,** 89–99.

Boorsma, A., Foat, B. C., Vis, D., Klis, F., and Bussemaker, H. J. (2005). T-profiler: scoring the activity of predefined groups of genes using gene expression data. *Nucleic Acids Res.* **33,** W592–W595.

Butenhoff, J. L., Chang, S. C., Ehresman, D. J., and York, R. G. (2009). Evaluation of potential reproductive and developmental toxicity of potassium perfluorohexanesulfonate in Sprague Dawley rats. *Reprod. Toxicol.* **27,** 331–341.

Calafat, A. M., Wong, L. Y., Kuklenyik, Z., Reidy, J. A., and Needham, L. L. (2007). Polyfluoroalkyl chemicals in the U.S. population: data from the National Health and Nutrition Examination Survey (NHANES) 2003–2004 and comparisons with NHANES 1999–2000. *Environ. Health Perspect.* **115,** 1596–1602.

Chateau-Degat, M. L., Pereg, D., Dallaire, R., Ayotte, P., Dery, S., and Dewailly, E. (2010). Effects of perfluorooctanesulfonate exposure on plasma lipid levels in the Inuit population of Nunavik (Northern Quebec). *Environ. Res.* **110,** 710–717.

Curran, I., Hierlihy, S. L., Liston, V., Pantazopoulos, P., Nunnikhoven, A., Tittlemier, S., Barker, M., Trick, K., and Bondy, G. (2008). Altered fatty acid homeostasis and related toxicologic sequelae in rats exposed to dietary potassium perfluorooctanesulfonate (PFOS). *J. Toxicol. Environ. Health A* **71,** 1526–1541.

de Groot, P. J., Reiff, C., Mayer, C., and Muller, M. (2008). NuGO contributions to GenePattern. *Genes Nutr.* **3,** 143–146.

de Haan, W., de Vries-van der Weij, J., van der Hoorn, J. W., Gautier, T., van der Hoogt, C. C., Westerterp, M., Romijn, J. A., Jukema, J. W., Havekes, L. M., Princen, H. M., *et al.* (2008a). Torcetrapib does not reduce atherosclerosis beyond atorvastatin and induces more proinflammatory lesions than atorvastatin. *Circulation* **117,** 2515–2522.

de Haan, W., van der Hoogt, C. C., Westerterp, M., Hoekstra, M., Dallinga-Thie, G. M., Princen, H. M., Romijn, J. A., Jukema, J. W., Havekes, L. M., and Rensen, P. C. (2008b). Atorvastatin increases HDL cholesterol by reducing CETP expression in cholesterol-fed APOE*3-Leiden.CETP mice. *Atherosclerosis* **197,** 57–63.

de Haan, W., de Vries-van der Weij, J., Mol, I. M., Hoekstra, M., Romijn, J. A., Jukema, J. W., Havekes, L. M., Princen, H. M., and Rensen, P. C. (2009). PXR agonism decreases plasma HDL levels in ApoE3-Leiden.CETP mice. *Biochim. Biophys. Acta* **1791,** 191–197.

D'eon, J. C., Hurley, M. D., Wallington, T. J., and Mabury, S. A. (2006). Atmospheric chemistry of N-methyl perfluorobutane sulfonamidoethanol, C4F9SO2N(CH3)CH2CH2OH: kinetics and mechanism of reaction with OH. *Environ. Sci. Technol.* **40,** 1862–1868.

Egusa, G., Brady, D. W., Grundy, S. M., and Howard, B. V. (1983). Isopropanol precipitation method for the determination of apolipoprotein B specific activity and plasma concentrations during metabolic studies of very low density lipoprotein and low density lipoprotein apolipoprotein B. *J. Lipid Res.* **24,** 1261–1267.

Ehresman, D. J., Froehlich, J. W., Olsen, G. W., Chang, S. C., and Butenhoff, J. L. (2007). Comparison of human whole blood, plasma, and serum matrices for the determination of perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and other fluorochemicals. *Environ. Res.* **103,** 176–184.

Giesy, J. P., and Kannan, K. (2001). Global distribution of perfluorooctane sulfonate in wildlife. *Environ. Sci. Technol.* **35,** 1339–1342.

Hansen, K. J., Clemen, L. A., Ellefson, M. E., and Johnson, H. O. (2001). Compound-specific, quantitative characterization of organic fluorochemicals in biological matrices. *Environ. Sci. Technol.* **35,** 766–770.

Haughom, B., and Spydevold, O. (1992). The mechanism underlying the hypolipemic effect of perfluorooctanoic acid (PFOA), perfluorooctane sulphonic acid (PFOSA) and clofibric acid. *Biochim. Biophys. Acta.* **1128,** 65–72.

Ikeda, T., Aiba, K., Fukuda, K., and Tanaka, M. (1985). The induction of peroxisome proliferation in rat liver by perfluorinated fatty acids, metabolically inert derivatives of fatty acids. *J. Biochem.* **98,** 475–482.

Kissa, E. (2001). In *Fluorinated Surfactants and Repellents*, Marcel Dekker, New York, NY.

Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., and Seed, J. (2007). Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicol. Sci.* **99,** 366–394.

Li, X., Catalina, F., Grundy, S. M., and Patel, S. (1996). Method to measure apolipoprotein B-48 and B-100 secretion rates in an individual mouse: evidence for a very rapid turnover of VLDL and preferential removal of B-48- relative to B-100-containing lipoproteins. *J. Lipid Res.* **37,** 210–220.

Lieder, P. H., Chang, S. C., York, R. G., and Butenhoff, J. L. (2009). Toxicological evaluation of potassium perfluorobutanesulfonate in a 90-day oral gavage study with Sprague-Dawley rats. *Toxicology* **255,** 45–52.

Manal, A., Abd El-Nasser, M. A., Shaaban, A. A., and Doha, Y. A. (2009). Toxicological effects of perfluoroalkyl acids on pregnant female mice. *Ass. Univ. Environ. Res.* **12,** 23–39.

Martin, M. T., Brennan, R. J., Hu, W., Ayanoglu, E., Lau, C., Ren, H., Wood, C. R., Corton, J. C., Kavlock, R. J., and Dix, D. J. (2007). Toxicogenomic study of triazole fungicides and perfluoroalkyl acids in rat livers predicts toxicity and categorizes chemicals based on mechanisms of toxicity. *Toxicol. Sci.* **97,** 595–613.

Miyake, J. H., Doung, X. D., Strauss, W., Moore, G. L., Castellani, L. W., Curtiss, L. K., Taylor, J. M., and Davis, R. A. (2001). Increased production of apolipoprotein B-containing lipoproteins in the absence of hyperlipidemia in transgenic mice expressing cholesterol 7alpha-hydroxylase. *J. Biol. Chem.* **276,** 23304–23311.

Moreau, A., Vilarem, M. J., Maurel, P., and Pascussi, J. M. (2008). Xenoreceptors CAR and PXR activation and consequences on lipid metabolism, glucose homeostasis, and inflammatory response. *Mol. Pharmacol.* **5,** 35–41.

Nelson, J. W., Hatch, E. E., and Webster, T. F. (2010). Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. *Environ. Health Perspect.* **118,** 197–202.

Olsen, G. W., Burris, J. M., Burlew, M. M., and Mandel, J. H. (2003). Epidemiologic assessment of worker serum perfluorooctanesulfonate (PFOS) and perfluorooctanoate (PFOA) concentrations and medical surveillance examinations. *J. Occup. Environ. Med.* **45,** 260–270.

Olsen, G. W., Burris, J. M., Ehresman, D. J., Froehlich, J. W., Seacat, A. M., Butenhoff, J. L., and Zobel, L. R. (2007). Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ. Health Perspect.* **115,** 1298–1305.

Olsen, G. W., Chang, S. C., Noker, P. E., Gorman, G. S., Ehresman, D. J., Lieder, P. H., and Butenhoff, J. L. (2009). A comparison of the pharmacokinetics of perfluorobutanesulfonate (PFBS) in rats, monkeys, and humans. *Toxicology* **256,** 65–74.

Pietzsch, J., Subat, S., Nitzsche, S., Leonhardt, W., Schentke, K. U., and Hanefeld, M. (1995). Very fast ultracentrifugation of serum lipoproteins: influence on lipoprotein separation and composition. *Biochim. Biophys. Acta* **1254,** 77–88.

Post, S. M., de Crom, R., van Haperen, R., van Tol, A., and Princen, H. M. (2003). Increased fecal bile acid excretion in transgenic mice with elevated

expression of human phospholipid transfer protein. Arterioscler. *Thromb. Vasc. Biol.* **23,** 892–897.

Post, S. M., de Roos, B., Vermeulen, M., Afman, L., Jong, M. C., Dahlmans, V. E., Havekes, L. M., Stellaard, F., Katan, M. B., and Princen, H. M. (2000). Cafestol increases serum cholesterol levels in apolipoprotein E*3-Leiden transgenic mice by suppression of bile acid synthesis. *Arterioscler. Thromb. Vasc. Biol.* **20,** 1551–1556.

Post, S. M., Groenendijk, M., Solaas, K., Rensen, P. C., and Princen, H. M. (2004). Cholesterol 7alpha-hydroxylase deficiency in mice on an APOE* 3-Leiden background impairs very-low-density lipoprotein production. *Arterioscler. Thromb. Vasc. Biol.* **24,** 768–774.

Rakhshandehroo, M., Hooiveld, G., Muller, M., and Kersten, S. (2009). Comparative analysis of gene regulation by the transcription factor PPARalpha between mouse and human. *PLoS One* **4,** e6796.

Reich, M., Liefeld, T., Gould, J., Lerner, J., Tamayo, P., and Mesirov, J. P. (2006). GenePattern 2.0. *Nat. Genet.* **38,** 500–501.

Ren, H., Vallanat, B., Nelson, D. M., Yeung, L. W., Guruge, K. S., Lam, P. K., Lehman-McKeeman, L. D., and Corton, J. C. (2009). Evidence for the involvement of xenobiotic-responsive nuclear receptors in transcriptional effects upon perfluoroalkyl acid exposure in diverse species. *Reprod. Toxicol.* **27,** 266–277.

Rensen, P. C., Herijgers, N., Netscher, M. H., Meskers, S. C., van Eck, M., and van Berkel, T. J. (1997). Particle size determines the specificity of apolipoprotein E-containing triglyceride-rich emulsions for the LDL receptor versus hepatic remnant receptor in vivo. *J. Lipid Res.* **38,** 1070–1084.

Seacat, A. M., Thomford, P. J., Hansen, K. J., Clemen, L. A., Eldridge, S. R., Elcombe, C. R., and Butenhoff, J. L. (2003). Sub-chronic dietary toxicity of potassium perfluorooctanesulfonate in rats. *Toxicology* **183,** 117–131.

Seacat, A. M., Thomford, P. J., Hansen, K. J., Olsen, G. W., Case, M. T., and Butenhoff, J. L. (2002). Subchronic toxicity studies on perfluorooctanesulfonate potassium salt in cynomolgus monkeys. *Toxicol. Sci.* **68,** 249–264.

Shipley, J. M., Hurst, C. H., Tanaka, S. S., DeRoos, F. L., Butenhoff, J. L., Seacat, A. M., and Waxman, D. J. (2004). Trans-activation of PPARalpha and induction of PPARalpha target genes by perfluorooctane-based chemicals. *Toxicol. Sci.* **80,** 151–160.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., and Vaccarino, V. (2009). Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. *Am. J. Epidemiol.* **170,** 1268–1278.

Storey, J. D., and Tibshirani, R. (2003). Statistical significance for genomewide studies. *Proc. Natl. Acad. Sci. U. S. A.* **100,** 9440–9445.

Tilton, S. C., Orner, G. A., Benninghoff, A. D., Carpenter, H. M., Hendricks, J. D., Pereira, C. B., and Williams, D. E. (2008). Genomic profiling reveals an alternate mechanism for hepatic tumor promotion by perfluorooctanoic acid in rainbow trout. *Environ. Health Perspect.* **116,** 1047–1055.

van der Hoogt, C. C., de Haan, W., Westerterp, M., Hoekstra, M., Dallinga-Thie, G. M., Romijn, J. A., Princen, H. M., Jukema, J. W., Havekes, L. M., and Rensen, P. C. (2007). Fenofibrate increases HDL-cholesterol by reducing cholesteryl ester transfer protein expression. *J. Lipid Res.* **48,** 1763–1771.

van der Hoorn, J. W., de Haan, W., Berbee, J. F., Havekes, L. M., Jukema, J. W., Rensen, P. C., and Princen, H. M. (2008). Niacin increases HDL by reducing hepatic expression and plasma levels of cholesteryl ester transfer protein in APOE*3Leiden.CETP mice. *Arterioscler. Thromb. Vasc. Biol.* **28,** 2016–2022.

Voshol, P. J., Rensen, P. C., van Dijk, K. W., Romijn, J. A., and Havekes, L. M. (2009). Effect of plasma triglyceride metabolism on lipid storage in adipose tissue: studies using genetically engineered mouse models. *Biochim. Biophys. Acta* **1791,** 479–485.

Westerterp, M., van der Hoogt, C. C., de Haan, W., Offerman, E. H., Dallinga-Thie, G. M., Jukema, J. W., Havekes, L. M., and Rensen, P. C. (2006). Cholesteryl ester transfer protein decreases high-density lipoprotein and severely aggravates atherosclerosis in APOE*3-Leiden mice. *Arterioscler. Thromb. Vasc. Biol.* **26,** 2552–2559.

Xu, L., Krenitsky, D. M., Seacat, A. M., Butenhoff, J. L., and Anders, M. W. (2004). Biotransformation of N-ethyl-N-(2-hydroxyethyl)perfluorooctanesulfonamide by rat liver microsomes, cytosol, and slices and by expressed rat and human cytochromes P450. *Chem. Res. Toxicol.* **17,** 767–775.

Xu, L., Krenitsky, D. M., Seacat, A. M., Butenhoff, J. L., Tephly, T. R., and Anders, M. W. (2006). N-glucuronidation of perfluorooctanesulfonamide by human, rat, dog, and monkey liver microsomes and by expressed rat and human UDP-glucuronosyltransferases. *Drug Metab. Dispos.* **34,** 1406–1410.

Zhou, J., Zhai, Y., Mu, Y., Gong, H., Uppal, H., Toma, D., Ren, S., Evans, R. M., and Xie, W. (2006). A novel pregnane X receptor-mediated and sterol regulatory element-binding protein-independent lipogenic pathway. *J. Biol. Chem.* **281,** 15013–15020.